CAN SEA WATER MAINTAIN THE BEAT OF THE HEART OF FRESH WATER ANIMALS?

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In 1900 Loeb[1] introduced the idea of physiologically balanced solutions, *i. e.*, "salt solutions which contain such ions in such proportions as to annihilate completely the poisonous effect which each constituent would have if it were alone in solution." On this basis blood, sea water and Ringer's solution are examples of balanced solutions. Loeb[2] was led to this idea by the observation that young *Fundulus*, which live in sea water, can also live in distilled water, while they die rapidly in a pure solution of sodium chloride (or any other salt) of the same osmotic pressure as sea water. Now we know that the life of the heart is sustained in blood and in Ringer's solution; the question arises is it equally well sustained in sea water that has been made isotonic with the blood. We have already a few facts in this regard. Rogers[3] found normal sea water to be an excellent sustaining fluid for the heart of the marine crab, and Garrey,[4] says "the mammalian muscle lives longer in isotonic sea water than in any other inorganic solution tested." Osterhout[5] experimenting with *Vaucheria sessilis*, a green alga common in running water, found it would grow well and live indefinitely in sea water with a concentration approximating 3/32 *M.* According to Van't Hoff the composition of sea water is as follows: 100 mols. NaCl; 2.2

[1] Loeb, J., "On the Artificial Production of Normal Larvæ from the Unfertillized Eggs of the Sea Urchin (Arbacia)," *Am. Jour. Phys.*, Vol. 3, 1900, p. 434. Also "Studies in General Physiology," p. 590.

[2] Loeb, J., "On Ion-proteid Compounds and Their Rôle in the Mechanics of Life Phenomena," *Am. Jour. Phys.*, Vol. 3, 1900, p. 327.

[3] Rogers, C. G., "The Effects of Various Salts upon the Invertebrate Heart," *Jour. Exper. Zoöl.*, Vol. 2, 1905, p. 237.

[4] Garrey, Walter E., "Twitchings of Skeletal Muscles Produced by Salt Solutions, etc.," *Am. Jour. Phys.*, Vol. 13, 1905, p. 186.

[5] Osterhout, W. J. V., "Extreme Toxicity of Sodium Chloride and its Prevention by Other Salts," *Jour. Biol. Chem.*, V ol. 1, 1905–6, p. 363.

mols. KCl; 2 mols. $CaCl_2$; 7.8 mols. $MgCl_2$; 3.8 mols. $MgSO_4$; reaction alkaline. Sea water thus differs from Ringer's solution in containing magnesium, while it differs from blood only in the excessive amount of magnesium present. This being the case it was determined to try its effect on the heart of the fresh water turtle.

The technique employed was essentially that used by physiologists in experiments of this nature. Strips of the ventricle were prepared in the usual way and attached by means of platinum hooks to a light aluminium lever magnifying about nine times. In order to have controls the strip was sometimes divided longitudinally, and sometimes transversely, as Martin[1] has shown there is no difference in the action of the strips (except the amplitude), whether prepared one way or the other. By dividing transversely strips are obtained containing fibers of both the anterior and posterior wall of the ventricle, which appeals to one as being advantageous for comparison. The lever was not weighted except when the whole heart was used, in which case a one gram weight was added just far enough from the fulcrum to about balance the weight of the heart. The lower end of the strip was attached to a glass rod bent at right angles, which served as a fixed point. Cylinders containing about thirty-five cubic centimeters of solution were used to immerse the strips. In the case of the sinus and auricles, they were separated completely from the ventricle by an incision in the auriculo-ventricular groove, and suspended in the same way as were the ventricular strips. The sea water used was taken from the Pacific Ocean at a point about a mile below the Cliff House, San Francisco. It had a freezing depression of 1.85. It was made isotonic by dilution with water distilled in glass, and its concentration determined from time to time by the freezing point. Twenty-eight c.c. of sea water in 100 gave the same depression as the Ringer's solution used for controls, which was made up according to the following formula: 100 mols. NaCl; 2 mols. KCl; 2 mols. $CaCl_2$; trace of $NaHCO_3$; all of $m/8$ concentration. Unless otherwise stated when "Ringer" is referred to, this solution is meant.

[1] Martin, E. G., "An Experimental Study of the Rhythmical Activity of Isolated Strips of Heart Muscle," *Am. Jour. Phys.*, Vol. 11, 1904, p. 103.

The turtles used were mostly those common to this locality (California), but a few were from Illinois — the *Emys Meleagris*.

It may be as well to begin by stating that the turtle's heart will remain alive as long in dilute sea water as in "Ringer." By "alive" is meant that condition of the heart in which, though quiescent it still responds to a stimulus by a contraction. Usually an induction current was used to ascertain if the heart would still contract. Individual hearts vary within such wide limits that in experiments on the whole heart controls were considered of such doubtful value that they were seldom used. The temperature also is a factor, for in warm weather bacterial decomposition soon sets in. In cool weather records of the ventricle have been obtained extending over one hnndred hours; but most of this work was done at higher temperatures and the hearts would not last as long either in sea water or in "Ringer." The auricles have given an uninterrupted series of contractions lasting eighteen hours or more, in cool weather.

When a strip of ventricle from a turtle's heart is immersed in isotonic sea water, it remains perfectly quiet indefinitely. Occasionally it will give a single spontaneous contraction, but it has been known to remain for twenty-four hours without giving a single beat. The shortest time in any of my experiments that it remained quiet was three hours and three quarters, when it gave one contraction, followed by two more eighteen hours later. Control strips in "Ringer" would invariably give single contractions or groups of contractions, separated by periods of quiescence, and were always more active than the corresponding strips in sea water. If, however, the strips were first treated with pure NaCl in $m/8$ concentration until "sodium chloride arrest" was induced, the difference was more marked. The strip transferred to "Ringer" usually recovered and gave a good series of contractions; while that transferred to sea water would revive for a few minutes and then become quiet, giving contractions of a very slow rate, or even none at all. That it was still capable of contraction was demonstrated by its response to stimulation with the induced current.

In striking contrast to the apical strips was the behavior of the auricles, always taken from the same heart for comparison. As

is well known when they are suspended the auricles contract spontaneously. When placed in dilute sea water the rate was always increased by several beats per minute, and they gave an uninterrupted series of contractions gradually diminishing in rate and amplitude, lasting from seven to twenty-eight hours. Usually when longer than twelve to eighteen hours, towards the last the contractions would be irregular, with pauses between groups. Control auricles in "Ringer" showed practically no difference. They too exhibited a slight increase in rate and gave the same characteristic series of contractions — the average being about fourteen hours in both "Ringer" and sea water, with the longest and the shortest records to the credit of the sea water. The following description of a few experiments will supply some details.

Exp. Jan. 28/'07; temperature 17° C. Strip *A* was placed in Ringer's solution. During the first four hours no contractions occurred. During the next four hours there were four series of rapid contractions, each series lasting from two to five minutes, with one half to one hour between. In ten hours from beginning the experiment the strip failed to respond to the induced current. Strip *B* was placed in dilute sea water. During ten hours it did not give a single contraction. At the end of this time it still responded feebly to the induced current. The auricle of this heart was suspended in dilute sea water and gave a fine series of contractions lasting about three hours. Before immersion the rate was sixteen per minute; after immersion it was eighteen, becoming slower toward the end of the three hours. This was the shortest series of contractions obtained. For some unaccountable reason both the ventricle and auricles gave out in a much shorter time than usual.

Exp. Feb. 1/'07; temperature 20° C. Two strips of the ventricle were prepared in the usual way. Strip *A* was put in Ringer's solution, and after a latent period of twenty-five minutes, gave a single vigorous contraction. It then remained quiet for twenty minutes, when it again began to contract, and gave a series of contractions lasting about ten hours, the rate varying from one to eight per minute. Twenty-two hours after beginning the experiment it was stimulated with the induced current, but gave no response. Strip *B* in dilute sea water remained perfectly

quiet for eleven hours. It was then stimulated with the induced current, and gave five vigorous contractions of maximum amplitude after which it was again quiescent. Twenty-two hours after beginning the experiment, it was again stimulated and gave a fairly good contraction of about one third the maximum in height. The solution was turbid indicating bacteria, and two hours later it failed to respond to stimulation. The auricle of this heart was placed in "Ringer" and gave a fine series of contractions lasting eleven and one half hours, with an initial rate of twenty, which was increased to twenty-four after immersion in "Ringer."

Exp. Feb. 4/'07; temperature 18° C. Two strips of the turtle's heart were prepared as usual. Both strips were put in *m*/8 NaCl in order to bring them to "sodium chloride arrest." Strip *A* began contracting after a latent period of fifteen minutes; its rate, five per minute, increasing to fifteen with gradually diminishing amplitude. At the end of two hours and fifteen minutes it ceased contracting. After remaining quiet for fifteen minutes it was transferred to "Ringer." It began at once to recover, but the contractions were small in amplitude, accompanied by a strong rise of tone. The rate was nine, increasing to fifteen per minute. Nine and one half hours from beginning the experiment, it responded to electrical stimulation by a very feeble twitch. Strip *B* began contracting after a latent period of fifteen minutes. Its rate was four per minute increasing to twelve. It came to "sodium chloride arrest" at the same time as did strip *A*, and was transferred to dilute sea water. It began contracting at once with a rate of nine per minute. After about fifteen minutes it ceased suddenly for five minutes and then began centracting feebly and in groups. It then settled down to a slow rate of one or two per minute, but with increased amplitude, and continued this for four and one half hours. It was stimulated with the induced current at the same time as was strip *A*, and gave a single contraction that was more vigorous than strip *A*, although it was still feeble. The auricle was contracting in the air at a rate of twenty per minute. After it was put in sea water the rate was twenty-four. It gave an uninterrupted series of contractions lasting about nine hours. After a long rest it recovered a little and gave some weak contractions. The duration of its irritabil-

ity was about nineteen hours, nine of which were spent in vigorous activity.

These three experiments were selected because they came in a series, and it so happens that it includes two of those that are the least typical of any. Exp. Jan. 28/'07 as has been said, lived for a much shorter time than usual, while in exp. Feb. 4/'07 the turtle was blind and sluggish; when the plastron was removed the heart was not contracting, nor did the auricles contract until they were suspended.

Exp. Feb. 8/'07; temperature 17–21° C. Two strips of the turtle's ventricle were prepared and exhausted in NaCl. Strip *A* was then transferred to dilute sea water. At the end of six minutes it began contracting vigorously with a much greater amplitude than in NaCl; at first with a rate of six per minute, but soon dropping to one in two or three minutes. In about fifteen minutes it ceased abruptly and remained perfectly quiet for about three quarters of an hour. It was then transferred to "Ringer," and in seven minutes began giving maximum contractions with a somewhat irregular rhythm; there would be a group of six to eight per minute, then a pause for half a minute and then another group. When it had been contracting in this way for one and one half hours it was again transferred to sea water. It was then registering on a twelve hour drum and the rate was not taken; but in fifteen minutes it again ceased abruptly and remained perfectly quiet for between five and six hours. Again transferred to "Ringer," it remained quiet for half an hour and then began to contract vigorously, but with gradually diminishing amplitude, and during the night it ceased. Next morning it failed to respond to stimulation. Strip *B* was used as a control. After NaCl exhaustion it was placed in "Ringer" instead of sea water, and recovered at once with a rate of sixteen gradually dropping to twelve per minute. The subsequent course of this strip was similar to strip *A*; when *A* was in "Ringer," *B* was in sea water and *vice versa*. When *A* was active in "Ringer," *B* was quiescent in sea water. The record of the auricles was lost after seven hours.

Having ascertained the influence of dilute sea water upon the different parts of the heart separately, the whole heart was sus-

pended by passing a hook through the apex below and one through the auricle above. In this way one can obtain a record of both the ventricular and the auricular contractions in their relations one to the other. The results of several experiments show that sea water favors the relaxation of the ventricle, while it has either no effect or a slightly stimulating one upon the auricles. The tracings show a more orderly sequence of auricular systole followed by ventricular systole, than in "Ringer," where the auricular systole has a tendency to fuse with the ventricular, owing possibly to incomplete relaxation of the ventricle. The question is being studied further in connection with the effects of magnesium on the heart; and as this paper is in the nature of a preliminary communication, the discussion of it will be left to a future date.

As the only practical difference between sea water and "Ringer" is the magnesium present in the former, it seems more than likely that the latter salt is responsible for the difference in the effect of these two solutions. As in the case of the marine crab, so in the turtle's heart, sea water rendered isotonic with the blood seems to be an excellent sustaining fluid; in other words sea water isotonic with the blood is a "physiologically balanced solution" for the turtle's heart. It is conceded that sea water contains magnesium in excess of that usually found in blood, and it is for that very reason it has proved useful in pointing the way for further research; but while it is found in the blood in the proportion of about two of magnesium to three or more of calcium (Hammarsten), the conditions are reversed in muscle and nerve, the proportion being about two of magnesium to one of calcium. One would therefore expect the quantity of magnesium to vary considerably at different times, and it is difficult to say what constitutes an excessive amount; an optimum has yet to be determined.

In conclusion it should be said that these results were to be expected in the light of those of Quinton,[1] who not only transfused dogs with isotonic sea water in quantities equal to the quantity of blood withdrawn, but also injected large quantities intravenously, without serious consequences. "Entre l'eau de

[1] Quinton, R., "L'Eau de Mer Milieu Organique," 1904, p. 160.

mer et le milieu vital du Vértebré, il y a physiologiquement identité."

Summary.

1. Strips of the ventricle of the turtle's heart will live as long in isotonic sea water as in Ringer's solution.

2. After "sodium chloride arrest," strips of ventricle will recover as well in isotonic sea water as in Ringer's solution.

3. The whole heart will beat as long in isotonic sea water as in Ringer's solution.

This problem was suggested to me by Professor Loeb, and I have to thank him for many helpful hints.

NOTE. — At the time these experiments were begun and the paper was in preparation, I was ignorant of the work of Mayer[1] on the embryo heart of the loggerhead turtle, which should be referred to.

[1] Mayer, Alfred G., "Rhythmical Pulsations in Scyphomedusæ," Carnegie Institution of Washington, Publication 47, 1906.